LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 60, 2015, 1212–1221

doi: 10.1002/lno.10094

Thermal adaptation, phylogeny, and the unimodal size scaling of marine phytoplankton growth

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Abstract

Studies on the size-scaling of phytoplankton growth rate are usually based on temperature-corrected growth rates or experiments performed at a fixed temperature, but the effects of differing thermal adaptation of small and large species have not been considered. We use an extensive dataset of phytoplankton growth rate responses to temperature and cell size to show that the unimodal size-scaling of phytoplankton growth depends strongly on temperature, and is not significant at high temperatures where the most common picophytoplankton species grow at their optimum. Furthermore, we show that the unimodality results from the different growth rate scaling of picophytoplankton, which differs phylogenetically from larger phytoplankton taxa. Using ribosomal RNA sequences we recalculated the size-scaling allometry with Phylogenetic Generalized Least Squares regression. After phylogenetic correction, the unimodal relationship is not significant at any temperature, suggesting that the observed curvature reflects the evolutionary adaptation of picophytoplankton to the warm conditions usually encountered in oligotrophic environments.

Metabolism is the basis of the energetic exchange between organisms and the environment. According to the metabolic theory of ecology (Brown et al. 2004), metabolic rates (M) scale with cell volume (BV) following a power-law of the form $M \propto aBV^b$, where a is a mass-independent normalization constant and b is the size-scaling exponent, which commonly takes a value of approximately 3/4 (Kleiber 1947). Hence, mass-specific metabolic rates, such as individual growth rate, should scale as $-1/4$ of the organism biovolume (Hemmingsen 1960; López-Urrutia et al. 2006). In marine phytoplankton, some studies have supported this theoretical scaling (Banse 1976; Blasco et al. 1982; Niklas and Enquist 2001; Edwards et al. 2012), although they are usually based on the study of one or two size classes (Banse 1976; Blasco et al. 1982). Indeed, the inclusion of a wider range of phytoplankton cell size, covering from picophytoplankton to large diatoms, leads to a weaker (Banse 1982; Sommer 1989; Chisholm 1992) or almost inexistent relationship between mass-specific growth rate and cell volume (Marañón et al. 2007; Litchman et al. 2007; Marañón 2008; Huete-Ortega et al. 2012). The controversy around the allometric scaling value has increased recently with the report of an unimodal relationship between mass-specific growth rate and size (Chen and Liu 2010, 2011; Marañón et al. 2013). This controversy has important implications for the function of the global ecosystem, as the choice of the value is essential to understand the factors that control the marine phytoplankton community size structure.

According to Chen and Liu (2011), the unimodality in the phytoplankton allometry can be mainly attributed to the lower growth rates by the smallest phytoplankton, specially the unicellular *Prochlorococcus* and *Synechococcus* (Chisholm 1992), but also some of the smallest eukaryotic species (Bec et al. 2008). As a result of evolutionary adaptation to oligotrophic regions, picophytoplankton seem to have suffered from a reduction in genome and cell size (Partensky and Garczarek 2010). This minimizes the resources necessary to live but at the cost of having lower growth rates. As cell and genome size is reduced, the proportion of essential, nonscalable cellular components (membranes and nucleic acids) increases, which leads to a reduction in the fraction of cytoplasm available for other scalable, catalytic components such as those involved in growth rate, tending to decrease growth rate (Raven 1998; Raven et al. 2013). Raven (1998) suggested that the unimodal relationship between growth rate and cell

Additional Supporting Information may be found in the online version of this article.

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size might be more a consequence of phylogenetic variations in the taxon-related constant *a* in the allometric equation rather than to changes in *b*.

The shared evolutionary history of related species establishes a correlation between data in allometric scaling studies that, if not accounted for, can result in biased scaling exponents (Capellini et al. 2010; Kolokotrones et al. 2010; Ehnes et al. 2011). Phylogenetic approaches are commonly used to deal with intraspecific and interspecific trait variability combining evolutionary relationships between species and correlations between traits (Felsenstein 1985, 2008; Housworth 2004; Ives et al. 2007; Connolly et al. 2008). But the inclusion of such phylogenetic approaches in studies of metabolic scaling has been controversial, with authors questioning their validity or utility (Björklund 1994; Ricklefs and Starck 1996; McNab 2008), arguing that phylogenetic correction does not significantly change the value of the estimated slope (reviewed in Glazier 2005) and others claiming it is necessary to provide these analyses (Blackburn and Gaston 1998; Garland et al. 1999). For terrestrial invertebrates, Ehnes et al. (2011) have shown that the inclusion of phylogeny removes the curvatures in allometric scaling models. In contrast, very few studies have applied phylogenetic approaches to the study of phytoplankton allometry (Connolly et al. 2008; Bruggeman et al. 2009; Bruggeman 2011).

Failures to detect unimodal allometric scaling have also been attributed to the lack of homogeneity in the data used. Marañón et al. (2013), in an effort to avoid the uncertainties associated with the analysis of data measured under different growth conditions, maintained a series of phytoplankton cultures at the same temperature ($18^{\circ}C \pm 0.5^{\circ}C$) and obtained a unimodal size scaling of phytoplankton growth rates. But each phytoplankton species has an optimum temperature at which its growth is maximum (Eppley 1972; Thomas et al. 2012). The selection of the temperature at which to perform the size scaling experiments might be nontrivial if optimum temperature and phytoplankton cell size are correlated.

In this work, we will assess whether a relationship between cell size and thermal optimum exists for marine phytoplankton. We will test the influence of temperature and the shared evolutionary history of species on allometric scaling of growth rate. Our final aim is to show that the curvature is the result of the evolutionary specialization of picophytoplankton to the warm conditions usually encountered in oligotrophic environments.

Material and methods

We used an extensive dataset of phytoplankton growth responses to temperature compiled by Thomas et al. (2012) for a total of 194 isolates/strains from estuarine and marine waters. These traits were estimated from >5000 growth rate measurements, synthesized from 81 studies between 1935 and 2011. This dataset only includes experiments where resources, such as light or nutrients, were not limited [details are provided in the supplementary information in Thomas et al. (2012)].

To explore the relationship between cell size, maximum growth rate, and temperature, we compiled cell volumes for each of the phytoplankton species in Thomas et al.'s (2012) dataset. Cell volumes were collected from the literature (Supporting Information Table S1). Cell sizes in the dataset ranged from 1.1×10^{-1} μm^3 to 2.5×10^5 μm^3 (0.59–78.16 equivalent spherical diameter (ESD)).

Our hypothesis relies on the fact that the curvature is the result of the picophytoplankton adaptation to oligotrophic conditions and thus to the warm temperatures that usually characterized these areas. Hence, this implies two different but at the same time interrelated effects: temperature and phylogeny. Here, we will test on one hand if the small species form a phylogenetic branch well separated from the larger ones. On the other hand, we will test how the growth rate measured at different temperatures may influence the size-scaling of growth for marine phytoplankton.

To test whether the shared evolutionary history of species influence the emergence of the unimodal pattern in size scaling of growth rates, we assessed the phylogenetic similarity between species using the ribosomal RNA (rRNA) gene as phylogenetic marker and calculating branch lengths between species in the phylogenetic tree. 18S (for eukaryotes) and 16S (for prokaryotes) rRNA sequences of the species in the compilation by Thomas et al. (2012) were retrieved from the GenBank database when available, resulting in a total of 121 isolates/strains. In those cases where a strain had not been sequenced, we selected another strain of the same species, assuming that the similarity between strains of the same species should be high. When a species had several thermal growth response curves recorded but the phylogenetic information was restricted only to one strain, we calculated an average thermal response for that species.

Phylogenetic analyses

To introduce the information provided by the phylogenetic tree into the allometric scaling analysis, a Phylogenetic Generalized Least Squares (PGLS) regression (Felsenstein 1985) was applied. Unlike standard linear regression, this accounts for the fact that data points might be correlated as result of shared evolutionary history. Under the assumption that the trait evolves randomly (e.g., Brownian motion), closely related species have closer trait values. The PGLS uses branch lengths in the phylogenetic tree to estimate the correlation between traits, thereby correcting the dependence of data points.

Alignment of RNA sequences to build the phylogenetic tree was done with MUSCLE (using default settings) through the *muscle* package (Edgar 2004) in *R* (R Development Core Team 2008). The ends of the alignment were manually

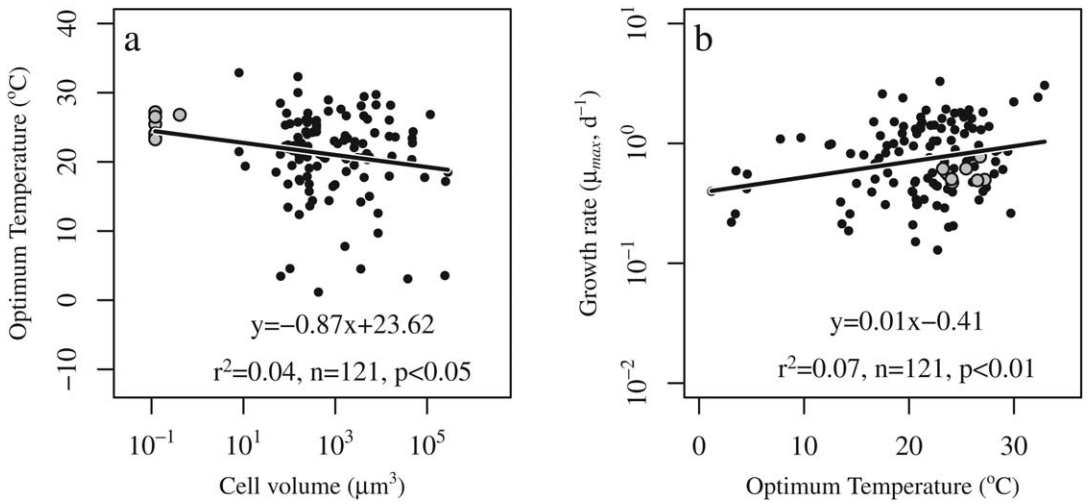

Fig. 1. Relationship between species optimum temperature and (a) cell size and (b) maximum growth rate. Gray dots show species composing the picophytoplankton.

trimmed. The tree was calculated using maximum-likelihood (ML) analysis carried out using PhyML v.3.1 (Guindon and Gascuel 2003), with the GTR+gamma+I model selected as the best tree using the Akaike information criterion (AIC) (Akaike 1974). Package *ape* (Paradis et al. 2004) was used to call these external applications from *R*.

Following the methodology described in Kolokotrones et al. (2010), we used Pagel's covariance structure (Pagel 1999) for the PGLS. This structure allows to account for covariance due to evolution including an extra parameter, λ. Although a Brownian motion (i.e., traits have evolved randomly) is assumed to model the variance of a particular species, the introduction of λ attenuates the correlation between species. This value is optimized during the fitting process and test for phylogenetic signal in the data taking values from 0 (phylogenetic independence between data) to 1 (original diffusion model with untransformed branch lengths). The PGLS was also applied using the *ape* package.

Size-scaling of growth rate and species thermal tolerance curves

We used the compilation of growth responses to temperature provided by Thomas et al. (2012) to fit the thermal tolerance curve of each species. We followed the same procedure as Thomas et al. (2012) and applied a maximum likelihood estimation using the *bbmle* package in *R* (R Development Core Team 2008). Using the thermal tolerance curve of each species, the optimum temperature was selected as the temperature at which growth rate is maximum.

These thermal tolerance curves provide estimates, for each species, of the growth rate at different temperatures. We calculated the size scaling of growth rate at 1°C intervals from 2°C to 33°C using for each species the predicted growth rate from the thermal tolerance curve. To avoid extrapolation problems that might bias the estimated growth rates at temperatures outside the range of the measured temperatures, we have restricted the predicted values to the temperature range where each species has been measured. When at the maximum (or minimum) temperature measured the growth is 0, we considered that the species will not grow above (or below) that temperature. However, if the measured growth rate at the extreme temperature is higher than 0, we removed any prediction outside that temperature as it would not be realistic. We did not include on the analyses those estimated growth rates lower or equal to 0, assuming that if a species has a negative (or 0) growth it cannot grow. Finally, linear and quadratic regressions were then applied to the log–log relationship between growth rate and cell size at each temperature, both with and without phylogenetic correction.

Results

The optimum temperature for growth and cell volume are correlated ($r^2 = 0.04$, $p < 0.05$, Fig. 1a). Species with a cell volume lower than 2 μm ESD (i.e., picophytoplankton species, which compose a total of 23 of the strains shown here) show maximum growth rates at temperatures higher than 22°C, while larger species (a total of 98 strains) have optimum temperatures for growth between 2°C and 33°C. The small species in Thomas et al.'s (2012) dataset are adapted to warm conditions whereas large phytoplankton species are more diverse regarding their optimum temperatures for growth with species with optima along almost the full ocean thermal range (Fig. 1a). Regardless of cell-size, and resembling Eppley's (1972) pattern, the maximum growth rate of species which have a growth optimum in warm conditions is higher than that of species with an optimum in colder environments. There is an exponential relationship between maximum growth rate of

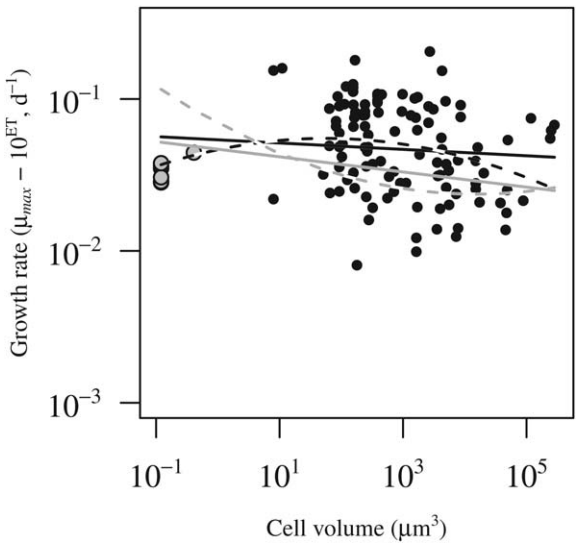

Fig. 2. Size scaling of growth for Thomas et al.'s (2012) data. Black solid and dashed lines correspond to a linear and quadratic fits, respectively. Gray solid and dashed lines correspond to a linear and quadratic PGLS fits, respectively. Regression equations are shown in Table 1. A common slope for temperature of 0.009 (calculated as the mean temperature slope for all analyses) was used to plot the relationship between temperature-corrected growth rate and cell size.

each species and optimum temperature (Fig. 1b, $\log_{10}(\mu)_{\max} = 0.013 \times T - 0.41$, $r^2 = 0.07$, $p < 0.01$).

The temperature dependence on the maximum growth rate should be removed to discern its effect from that of size in the analysis of the size-scaling of growth. For this reason and to correct the temperature effect, we used a multiple regression including temperature and cell size as predictors (Fig. 2). The quadratic term in the relationship $\log_{10}(\mu)_{\max} = \text{Temp} + \log_{10}(\text{BV}) + \log_{10}(\text{BV})^2$ is significant ($r^2 = 0.12$, $p < 0.01$) and the quadratic model is a better predictor than the linear model ($r^2 = 0.07$, $p = 0.014$, Δ AIC = 5.5, Fig. 2). This unimodal pattern is mainly due to the picophytoplankton species having lower than average growth rates.

We replicated these analyses applying a PGLS regression to the dataset. The quadratic term is no longer significant ($\lambda = 0.95$, $p = 0.205$, Δ AIC = 6.49) and the linear fit ($\lambda = 0.94$, $p = 0.058$) is now a better predictor. The relationship between temperature corrected growth rate and cell size was not significant for Thomas et al.'s (2012) data. In addition, λ values were close to 1 for all analyses revealing a strong phylogenetic signal in the data. This implies that differences in the species growth rate are correlated with the phylogenetic distance between species. These results suggest that the observed curvature in the size scaling of growth rate is a consequence of the shared evolutionary history. Conversely, the temperature slope was different for nonphylogenetical and PGLS analyses (Table 1), what suggest a phylogenetic effect on the different thermal adaptation of phytoplankton species.

Up to this point, we have evaluated the combined effects of temperature and phylogeny on the allometric scaling of growth rates. This is the common practice when data are compiled for different species measured at different temperatures. The alternative way to analyze the size scaling of growth is to measure the growth rates of a set of species at the same temperature (e.g., Marañón et al. 2013). With the growth vs. temperature growth curves, we can simulate such experiments at different temperatures. For each temperature, we estimate the growth rate of each species and use that data to analyze the size scaling. For example, Fig. 3a represents the growth estimates at 30°C. If we calculate with the data for all species (gray circles) the size scaling, the quadratic term is not significant (Quadratic model: $r^2 = 0.04$, $p = 0.153$; linear model: $r^2 = 0.003$, $p = 0.68$). Similarly, at 12°C we can plot the predicted growth rates for each species (Fig. 3b) but here the quadratic term is significant and better predictor than the linear one (Quadratic model: $r^2 = 0.18$, $p < 0.001$, AIC = 83.57; linear model: $r^2 = 0.06$, $p = 0.02$, Δ AIC = 10.8). We can repeat this process at 1°C intervals from 2°C to 33°C and calculate the significance of the quadratic term (column "p value" in Fig. 3c) for each temperature. We estimate the growth rate of each species and use all data to

Table 1. Parameters for the size scaling relationships of phytoplankton growth rate using multiple linear and quadratic regressions with both size and temperature as predictors. Results for both nonphylogenetical and PGLS analyses are shown. Linear model: $\log_{10}(\mu)_{\max} = a\text{Temp} + b\ \log_{10}(\text{BV}) + d$. Quadratic model: $\log_{10}(\mu)_{\max} = a\text{Temp} + b\ \log_{10}(\text{BV}) + c\ \log_{10}(\text{BV})^2 + d$. Number of data points was 121 for all analyses.

Non-PGLS	*a*	*b*	*c*	*d*	r^2	*p*	AIC
Linear	0.012±0.005	−0.018±0.021		−0.338±0.125	0.07	0.014	56.05
Quadratic	0.01±0.004	0.088±0.044	−0.024±0.009	−0.408±0.124	0.12	<0.01	50.55

PGLS	*a*	*b*	*c*	*d*	λ	*p*	AIC
Linear	0.005±0.004	−0.058±0.03		−0.176±0.474	0.94	0.058	45.62
Quadratic	0.005±0.004	−0.219±0.13	0.025±0.019	−0i.015±0.52	0.95	0.205	52.11

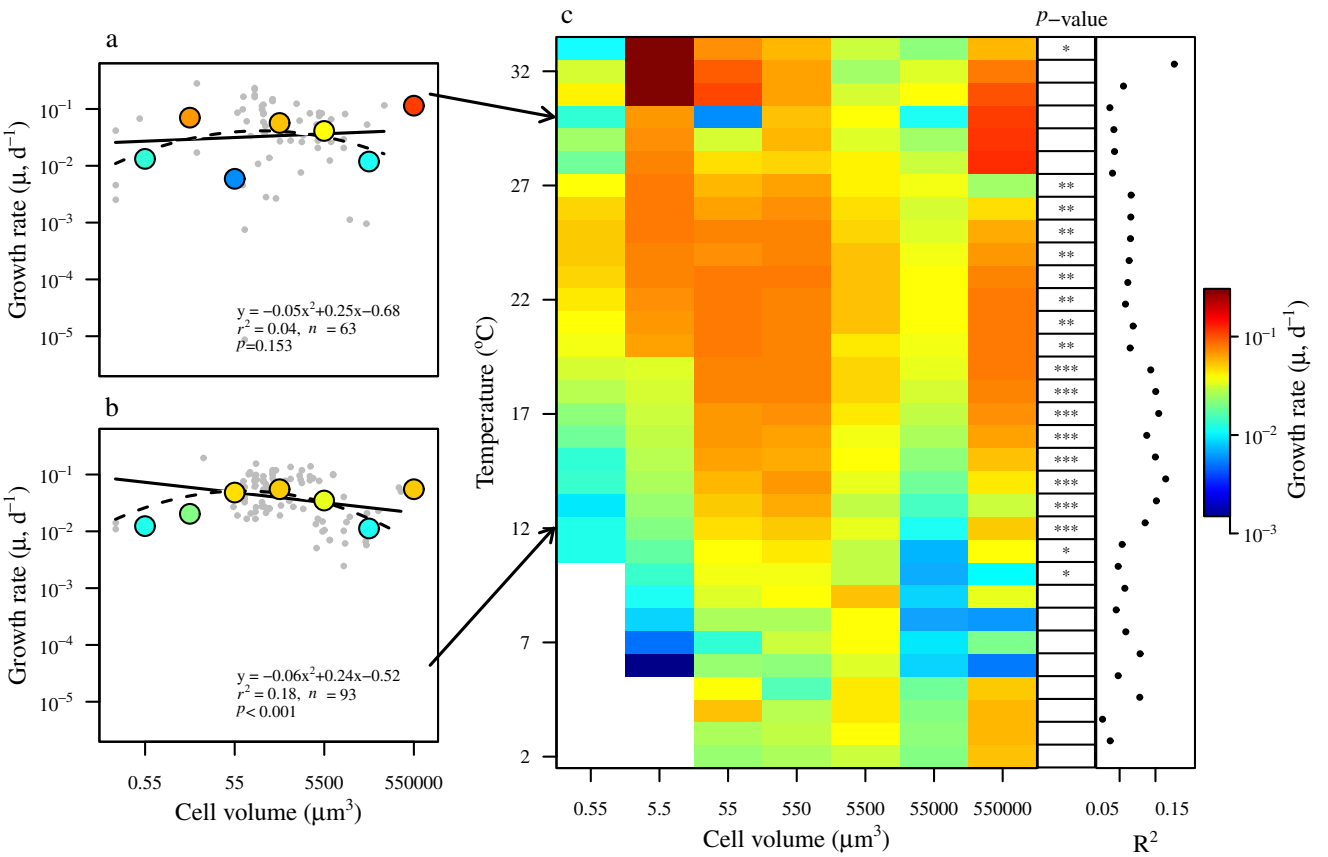

Fig. 3. Effect of temperature on the size scaling of growth rate. Panels on the left show the size scaling of growth ($\log_{10}$ transformed) for predicted growth rates at (a) 30°C and (b) 12°C. Gray dots show all data points whereas colors dots show the corresponding averaged growth rates for each size bin as used in the color matrix plot (c). The black solid line corresponds to a linear fit. The black dashed line corresponds to a quadratic fit. Linear fit in (a): $\log_{10}(\mu) = 0.03 \times \log_{10}(BV) - 0.55$; ANOVA: $r^2 = 0.003$, $n = 63$, p-value = 0.68. Linear fit in (b): $\log_{10}(\mu) = -0.09 \times \log_{10}(BV) - 0.16$; ANOVA: $r^2 = 0.06$, $n = 93$, p-value = 0.019. Quadratic fits are shown in the panels. (c) The color matrix shows for each temperature from 2°C to 33°C (y axis) the averaged growth rate at each cell size bin (x axis). The p-value column shows the degree of significance of the quadratic fit for the $\log_{10}$-$\log_{10}$ relationship between growth rate and cell size using all data points (no data binning). When the quadratic term is not significant, i.e., p-value > 0.05 the box appears empty. (*) indicates p-value < 0.05, (**) indicates p-value < 0.01 and (***) p-value < 0.001. The right panel shows the ratio between the r-squared of the quadratic term and the r-squared of the linear term for the different fits at each temperature, i.e., the proportional increase in explained variance of the quadratic fit in relation to the linear fit.

analyze the size scaling (i.e., no binning is used here). The unimodal growth rate scaling does not occur at the extremes of the thermal range. At the highest temperatures, the growth rates of picophytoplankton are not significantly lower than those of nanophytoplankton and microphytoplankton. The quadratic term was not significant at low temperatures, but we lack growth data for small species in this thermal range, i.e., below 10°C for picophytoplankton and below 6°C for nanoplankton. At temperatures from 10°C to 27°C, the unimodal scaling of phytoplankton growth rate is significant and contributes to explain a significant amount of the variance (right panel in Fig. 3c). A significant unimodal relationship appears at 33°C. We think this could be due on one hand to the scarcity of data measured at extreme temperatures and, on the other hand, to have been restricted the database to only those species that have been sequenced. When the whole dataset is used, the significance of the quadratic fit is restricted from 11°C to 26°C (see Supporting Information Fig. S1). Nevertheless, when a PGLS is applied to the size scaling of growth rate at each temperature, and hence the shared evolutionary history of species is taken into account, the curvature is no longer significant at any temperature, supporting the hypothesis of its evolutionary origin. The color matrix plot in Fig. 3c summarizes these results. For each temperature degree, we split the cell size range into seven different classes and calculated the average growth rate for each cell size bin. These averaged growth rates are shown on Fig. 3a,b as color points. Notice that binning was used only for visualization and was not taken into account on the analyses. The different number of species within each size bin may bias the average values. For instance, only two species comprise the second size bin, so a

higher number of species would help to get a more accurate result. Our results show a clear pattern where as we move toward higher temperatures the curvilinear scaling disappears. The unimodality on the relationship between cell size and growth rate depends strongly on temperature and it is not significant from ~27°C upward, i.e., where picophytoplankton grows at their optimum temperatures.

Discussion

The role of the evolutionary history of species on allometric scaling of marine phytoplankton has hardly been considered explicitly. We have evaluated the causes of the unimodal relationship between mass-specific growth rate and cell size (Chen and Liu 2011; Marañón et al. 2013). We have used PGLS regression (Felsenstein 1985) to understand the evolutionary effects on the linear and quadratic fits. Our results show that the quadratic/unimodal relationship is not significant after the phylogenetic correlation in the data is taken into account.

The curvature in the scaling relationship between mass-specific growth rate and cell size is mainly due to the inclusion of prokaryotic picophytoplankton in the analysis, but also to some picoeukaryote cells (Bec et al. 2008). When we compare the growth rate of phytoplankton species at their thermal optimum (Fig. 2 and Supporting Information Fig. S2), picophytoplankton have lower growth rates than larger phytoplankton but when phylogenetic correction is used, these lower growth rates are not explained by size. The same explanation has been found to occur in the dataset given by López-Urrutia et al. (2006) as analyzed by Chen and Liu (2011) (*see* Supporting Information Fig. S3). Chen and Liu (2011) suggested that the unimodal pattern may be the result of evolutionary adaptation of picophytoplankton to nutrient availability in oligotrophic environments. This was pointed out originally by Raven (1998), who suggested that the reduction in size in picophytoplankton increases the availability of resources at low nutrient levels but at the cost of a reduction in the proportion of scalable components devoted to cell growth. In addition, marine picocyanobacteria such as *Prochlorococcus* or *Synechococcus* form a phylogenetic branch separated not only from larger phytoplankton taxa but also from larger species within the cyanobacteria group (Supporting Information Fig. S4). Specially, *Prochlorococcus* has suffered an extensive genome streamlining that has affected most lineages at different proportions (Palenik 1994; Urbach et al. 1998; Rocap et al. 2002; Penno et al. 2006). Hence, the high variability of growth rates exhibited within the *Prochlorococcus* group (Fig. 1b) seems to correspond to different levels of genome streamlining rather to be a consequence of its tiny cell size (Partensky and Garczarek 2010). Recent studies suggest that both genome and cell size are mutually correlated (Ting et al. 2007) and, therefore, they have decreased concurrently during evolution as an adaptative feature to profit from the surrounding conditions (Partensky and Garczarek 2010).

This interrelation between phylogeny, size, and growth rate is evidenced by the strong phylogenetic signal in our data ($\lambda > 0.9$). The growth rate and size estimates for the different species are not independent: closely related species have growth rates and sizes more similar than species selected at random. The independence of data is one of the assumptions of conventional methods for data analysis and its violation might have various consequences, from biases in the regression coefficients to severe underestimation of uncertainties related to these values. Hence, if instead of using a phylogenetic correction as we do, all observations were treated as independent (Tang 1995; Finkel 2001; López-Urrutia et al. 2006; Litchman et al. 2007), a biased association may be observed between growth rate and cell size. However, phylogenetic regressions have also been criticized mainly for two reasons: first, because these methods attribute to ecology the remaining variation in character after phylogenetic correction, giving thus priority to the latter over ecology when, actually, they are not mutually exclusive because of the phylogenetic niche conservatism (Harvey and Pagel 1991; Freckleton et al. 2002). Second, phylogenetic regressions imply the validity of a "Brownian motion" to explain the constant rate of variability through the different branches of the phylogeny, which is not always appropriate. Yet, when a strong phylogenetic signal is apparent, as is the case here, we argue that accounting for the shared evolutionary history of species is essential to avoid biased conclusions due to the nonindependence in the data (Martins and Garland 1991; Bruggeman 2011). In the literature, in those cases where the curvature in metabolic scaling has been found to be relevant (e.g., Kolokotrones et al. 2010), the quadratic term was found significant after phylogenetic correction, which warrants an interpretation of the curvature independent of the evolutionary history of species.

The relevance of the inclusion of picophytoplankton is evident in previous studies which only considered larger species and reported linear exponents (Banse 1982; Sommer 1989; Finkel 2001; López-Urrutia et al. 2006; Litchman et al. 2007). It has been shown that the value of the linear exponent depends on the size range considered (*see* Fig. 2 in Chen and Liu 2011). For instance, the difference in the $-1/4$ exponent observed by López-Urrutia et al. (2006) and the slope of 0.03 obtained in Supporting Information Fig. S3 is that the former study only considered data where both phytoplankton volume and growth rate were measured in the same experiment. For Supporting Information Fig. S3, we have also used volume estimates measured for the same species in other studies, which extends the size range to picophytoplankton and substantially reduces the size scaling slope. This low slope is apparent using either cell volume (Supporting Information Fig. S3) or carbon biomass (*see* Fig. 1b in Chen and Liu 2011). Our analysis for the Thomas et al.'s (2012) compilation, where the slope is also lower

than 0.1, support that the size-scaling of marine phytoplankton departs significantly from the predicted −1/4 power rule (Marañón et al. 2007) and mass-specific growth rate scales independent of body volume when a large range size is considered (Marañón et al. 2013).

These confounding effects of phylogeny, size, and growth rate are further increased when we also consider that temperature affects both growth rates and phytoplankton cell size, and complicated by the evolutionary adaptation of picophytoplankton to warm environments. To correct for the effects of temperature on growth rate when data are compiled for species growing at different temperatures, an exponential relationship between temperature and growth is used to standardize the growth rates of all species to the same temperature. However, this correlation (Fig. 1b) and therefore the assumed exponent for the temperature correction might be biased by the fact that cell volume is also correlated to temperature (Fig. 1a) and picophytoplankton species (theoretically with lower growth rates) are predominantly present at the highest temperatures. As we show here, when a PGLS regression is applied the coefficient for the temperature correction changes, what suggest that this different thermal adaptation of picophytoplankton is also the result of the shared evolutionary history of species. The choice of the thermal dependence exponent might introduce some bias in the size scaling analysis (Sal and López-Urrutia 2011). A priori, this caveat might be avoided measuring the growth rate of all species under study at the same temperature. But, paradoxically, our results show that the size scaling of phytoplankton growth rates is largely dependent on the temperature at which growth rates are measured. For instance, the non-phylogenetically corrected unimodal scaling of phytoplankton growth rate is significant from 10°C to 27°C, but not at higher or colder temperatures. Hence, our results support the unimodality at 18°C reported by Marañón et al. (2013) but we add the perspective that, if growth rates were measured at different temperatures the size scaling might have differed.

These results are due to the adaptation of picophytoplankton to warm temperatures, characteristic of oligotrophic conditions, where their higher surface to volume ratio makes them advantageous for resource acquisition. Larger species, dominant in eutrophic environments, have a more diverse thermal preference and may have optimum temperatures along the full ocean thermal range (Fig. 1b). At warm temperatures, the picophytoplankton species in Thomas et al.'s (2012) compilation are all at their thermal optimum. Nanophytoplankton and microphytoplankton species either have their optimum at high temperatures, or are adapted to temperate conditions but are, nevertheless, able to grow at higher temperatures. The inclusion of data of species out of their thermal optimum results in a different pattern in Fig. 3a than in Supporting Information Fig. S1 where only species that have their thermal optimum at high temperatures are considered. The optimum temperature of the species seems to be the result of evolutionary adaptation to the environmental conditions they experience locally (Thomas et al. 2012). As picophytoplankton, specially *Prochlorococcus* strains, are usually most abundant in the warm oligotrophic waters (Flombaum et al. 2013), they are expected to have optimum growth at high temperatures. However, this does not mean that there are no picophytoplankton in cold environments. Chen et al. (2014) argued that there is not enough evidence to prove that picophytoplankton in general prefer warm conditions. Except *Prochlorococcus*, other picophytoplankton do have a wide geographic distribution (Li et al. 2009; Flombaum et al. 2013). But even those picophytoplankton species being able to grow at low temperatures (<10°C), such as some strains of *Synechococcus*, have optimum for growth at higher temperatures (Pittera et al. 2014). Failure to have an optimum does not imply it cannot grow at low temperatures. Until now, only one isolate of *Micromonas pusilla*, has been found to grow optimally at 6–8°C (Lovejoy et al. 2007). It is a psychrophilic Artic endemic ecotype that differs from other isolates of this genus showing higher optimum temperatures (Throndsen 1976). More data of isolates from cold environments should be needed to support our conclusions and see if the optimal temperature of picophytoplankton distributes along the full thermal range, as occur for larger species, or not. Similarly, we admit that the very few number of picophytoplankton observations, in part due to the insufficient number of sequences, could mask the significance of the quadratic term. There is hence a clear need to obtain more thermal response curves for the growth rate of other picophytoplankton species.

Our temperature simulation experiment combines the estimation of thermal reaction norms to predict the growth rate of each species at different temperatures and the analysis of size scaling at each temperature. Ideally, these results should be confirmed experimentally by making a full experimental design where both temperature responses and size-scaling experiments are performed in parallel. But, the number of treatments in such a factorial design would make the study almost impractical. Community wide attempts (Boyd et al. 2013) might be the solution to fully test our hypothesis. Although the collation of data in Thomas et al. (2012) that we used to estimate the thermal reaction curves comes from a wide range of experimental protocols, a recent comparison with the dataset from such a community-wide study (Boyd et al. 2013) found slight differences on the maximum growth rate of species, but optimum temperatures and thermal reaction norms were similar across studies.

To construct a simple model including all the effects of size, phylogeny, and temperature on growth rate is still hard. A complicated model, although comprehensive, is of little use in biology-physics coupled models. Perhaps from another perspective, the low R^2 in the size-scaling models of phytoplankton specific growth rate suggests that the unimodal pattern is trivial even if it does exist. The phylogeny-dependent and

isometric growth rate model might be a good model that can be used in large-scale modeling exercises.

Although our results reveal that the unimodal scaling depends on temperature, the role of phylogeny seems to be much more important. Even at low temperatures, where picophytoplankton shows very low growth rates, a curvature appears to be non significant after phylogenetic correction. In summary, our results state that the allometric slope of phytoplankton growth rates is variable and do not consistently support a specific theoretical value when a large range of cell sizes are included. The strong phylogenetic signal exhibited in our data reveals that phylogeny should be borne in mind in allometric studies, as variability on the species growth rates seems to be consequence of a common evolutionary history rather than uniquely an effect of their size. This supports Raven's (1998) hypothesis that picophytoplankton have lower growth rates in an effort to increase the efficiency of resources acquisition at low nutrient levels. Adaptations such as the latter have been a common feature along the evolutionary history of organisms. The transition across major evolutionary groups, such as from prokaryotes to unicellular eukaryotes, has been followed not only by increases in size but also by structural and functional innovations in order to overcome existing constrains. Each evolutionary group displays a different scaling that reflects the way in which metabolic rates are limited by either the number of complexes where ATP synthesis occur or by changes on cell surface area that affects resource supply rates. As cell size increases and these limitations come true, new metabolic designs have allowed to overcome them, giving rise to a different size-scaling (DeLong et al. 2010). This shift in metabolic scaling from prokaryotes to eukaryotes (DeLong et al. 2010) thus would be in accordance with the unimodal scaling of phytoplankton growth due to the different growth rate scaling of prokaryotic picophytoplankton.

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Acknowledgments

This work was partially supported by projects METabolic OCean Analysis (METOCA) funded by Spanish National Investigation+Development+Innovation (I+D+I) Plan and Modelado de las reglas de ensamblado y estabilidad de los ecosistemas de comunidades planctónicas en el océano global (MARES - CGL2013-41256-P). Financial support was also provided by the Principado de Asturias FEDER (GRUPIN14-144) S.S. was funded by a Formación de Personal Universitario (FPU) grant program from Spanish Ministry of Education (MEC). F.G. was funded by a Formación de Personal Investigador (FPI) grant program from Spanish Ministry of Economy and Competitivity (MINECO).

Submitted 30 October 2014
Revised 22 February 2015
Accepted 4 March 2015

Associate Editor: Dr. Heidi Sosik